THE SECRETION OF INVERTASE BY PLANT ROOTS

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In an earlier paper (Knudson, 3) on the utilization of certain carbohydrates by green plants, the observation was repeatedly made that reducing sugars appeared in culture solutions containing sucrose. In discussing the possibility of invertase secretion the following statements were made:

"It has not yet been definitely proved that the inversion of saccharose is due to invertase secreted into the culture solution. It is possible that the saccharose is inverted in the roots and the reducing sugars are secreted, but this is less probable. It is possible also that the enzyme may be released as a result of the death of root hairs or other cells of the root and that it is not secreted from living cells."

A few observations have been made by other investigators on this subject of enzyme secretion, but the observations have been only incidental to other investigations and the few reports are conflicting. It seemed desirable therefore to investigate thoroughly the possibility of enzyme secretion and particularly that of invertase, since it is this enzyme that is most likely to be found in the roots of plants. Accordingly the investigation here reported was undertaken. Not all the experiments performed are reported, but those omitted are in agreement with the results here given.

Methods

The methods employed are essentially the same as those used by Knudson and Smith (4) in their experiments on the secretion of amylase. The plants were grown in water cultures under sterile conditions, that is, with the root system in a nutrient medium free of microorganisms and the seed removed from contact with the culture solutions. The type of culture is shown in figure 1. The details of manipulation are sufficiently described by Knudson and Smith (4) and need no repetition here.

Pfeffer's nutrient solution was used, with the substitution, however, of dibasic potassium phosphate for the monobasic potassium phosphate. The solution was prepared according to the following formula: $Ca(NO_3)_2$ 4 grams, K_2HPO_4 1 gram, KNO_3 1 gram, $MgSO_4 \cdot 7H_2O$ 1 gram, KCl 0.5 gram, $FeCl_3$ 50 milligrams, distilled water 6 liters. To this solution was added, when desired, sucrose.

In the use of Pfeffer's solution, according to the formula given, it is essential that precautions be taken to prevent acidification of the culture solution. When the nutrient solution is sterilized in an autoclave for a period of 30 minutes or more, there may result an acid solution. This appears to be due to the reaction between calcium nitrate and dibasic potassium phosphate, whereby there is produced some tri-calcium phosphate

with the liberation of some nitric acid. If the sucrose is present, therefore, in the nutrient solution, a considerable portion may be inverted. The period of sterilization seems to be a factor in this acidification, for in some of the preliminary experiments no such inversion occurred when the entire nutrient solution was sterilized at one time.

Throughout the experiments here reported the following method was adopted. The nutrient solution was made up in two portions. Portion *A*

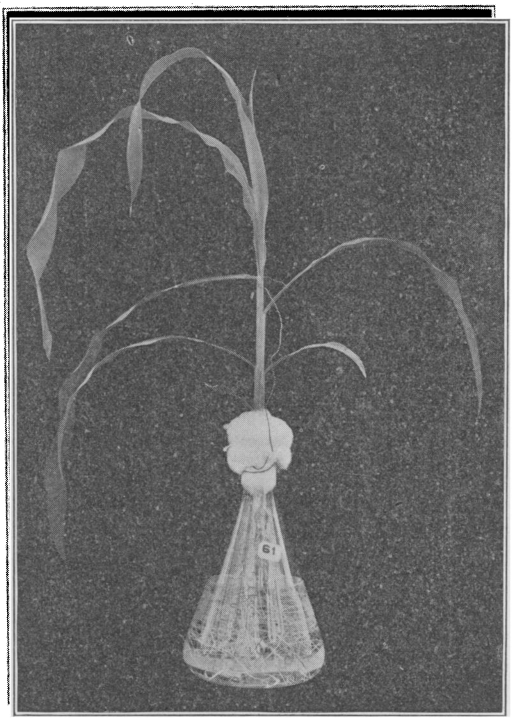

FIG. 1. See text for explanation.

includes all the salts except calcium nitrate. Portion *B* includes calcium nitrate only. To solution *A*, which is slightly alkaline, the sucrose was added. The solutions were originally made up double strength so that equal quantities of *A* and *B* would give the desired concentration of the sucrose and salts necessary for the nutrient solution. The method of procedure is as follows: When 1100 cc. of the culture solution is desired, 550 cc. of each solution (*A* and *B*) is accurately measured out. Solution *A* is placed in the culture flask and this is provided with a cotton stopper with a central tube. Through this tube is inserted the stem of a 9-centimeter funnel, and the funnel and neck of the flask are then covered with cotton to prevent any contamination after sterilization. Solution *B* is placed in a liter flask stoppered with cotton and the stopper and neck are also enclosed

with cotton. The two flasks are then sterilized in an autoclave for 30 minutes at a pressure of 15 pounds. When the solutions are cool, solution *B* is poured into the culture flask containing solution *A*. This transference of solution *B* to *A* takes place under conditions to minimize as much as possible the possibility of contamination. The funnel is then replaced by a cotton stopper and the cotton stopper and neck of the flask are covered again with cotton to prevent organisms and spores from lodging in the cotton stopper, circumstances which might cause contamination when the seedling is transferred to the culture flask. The flasks are permitted to stand several days before the seedlings are transferred, and at the time of transplanting any that show contamination are rejected.

Hydrogen-ion determinations were made by the indicator method, using mixtures of monobasic potassium phosphate and dibasic sodium phosphate prepared according to the methods of Soerensen (Prideaux, 7). These determinations were made at the outset of the experiment and also at its conclusion, and in some experiments mentioned subsequently the reaction of the culture solution was followed by adding to the culture solution the indicator, neutral red. The hydrogen-ion coneentration is expressed as the logarithm (the base being 10) of the normality with respect to the hydrogen ions. The minus sign is understood; for example, P[H]7 refers to a hydrogen-ion concentration of 10^{-7} normal.

Sugar determinations in experiment 1 were made by Kendall's method (2), and the reducing sugar is expressed as invert sugar. In all the other experiments the volumetric method of Cole (1) was used. This method proved to be a rapid and accurate method for the purpose. The reducing sugars are expressed as glucose. In the use of both methods the solutions were standardized against prepared sugar solutions, the sugars used being of a very high degree of purity.

Experiments

Experiment 1. In this experiment Canada field pea (*Pisum arvense* L.) was used. The culture vessels were pyrex flasks of one-liter capacity and the quantity of the nutrient solution was 1050 cc. Sterilization of the solutions was effected by autoclaving at 15 pounds pressure for 30 minutes. Seeds were sterilized by the use of calcium hypochlorite for one hour. The plants were grown in a greenhouse at an average temperature of 70° C.

At the conclusion of the experiment the culture solutions were tested for sterility by plating 1 cc. of each solution on an agar medium of Pfeffer's solution plus 1 percent sucrose. Only those cultures that proved to be sterile were analyzed. The results follow in table 1 and a typical culture is shown in figure 1.

There is in each of the culture solutions containing sucrose an appreciable gain in reducing sugars, but the gain is relatively slight as compared to the total amount of sucrose present. If the enzyme invertase is secreted, why

TABLE I. *Canada field pea. Duration, Nov. 2 to Dec. 13, 1916: 42 days.*

Culture Solution	Culture Number	Water Transpired (Cubic Centimeters)	Dry Weight			Total Sugar in Culture Solution at End of Experiment, Calculated as Invert Sugar (Grams)	Sugar Absorbed by Plant, Calculated as Invert Sugar (Grams)	Reducing Sugar in Culture Solution at End of Experiment, Calculated as Invert Sugar (Grams)	Gain in Reducing Sugar, Calculated as Invert Sugar (Grams)
			Roots (Grams)	Tops (Grams)	Total (Grams)				
Pfeffer's + ½ per cent sucrose	1	210	0.130	0.375	0.505	4.584	0.216	0.541	0.233
" + ½ per cent "	2	230	0.170	0.368	0.538	4.544	0.256	0.600	0.292
" + ½ per cent "	3	170	0.082	0.215	0.297	4.652	0.148	0.497	0.189
" + ½ per cent "	4	120	0.140	0.140	0.280	4.572	0.228	0.491	0.183
" + ½ per cent "	5	150	0.100	0.280	0.380				

is there not a greater production of reducing sugars? The maximum increase in reducing sugar is only one fifteenth of the sucrose present.

Circumstances prevented at this time any incubation experiment with the culture solutions to determine whether or not there would result an increase in reducing sugars which might be taken as evidence of the presence of invertase.

Experiment 2. The culture methods and conditions were essentially like those of the preceding experiment. The nutrient solution was slightly modified by the substitution of ferrous chloride for ferric chloride, and the sucrose used was Merck's highest purity. The nutrient solution at the outset had a hydrogen-ion concentration of P[H] 6.80.

Two plants were used in the experiment: corn, variety Weber's Dent, and Canada field pea. Unfortunately most of the cultures of Canada field pea became contaminated, and data were obtained from only one sucrose culture.

An examination of table 2 reveals the fact that as usual a better growth is obtained with sugar than without. An exception is culture number 6, which was maintained in diffused light in the laboratory for ten days preceding the conclusion of the experiment.

In cultures 6 to 10 inclusive there was noted an increase in reducing sugars, but none was found in cultures 11 to 15 inclusive. The amount of reducing sugar in the sucrose cultures, while appreciable, is again relatively small compared to the total sugar present. In culture number 8 the unusually large amount of reducing sugar was undoubtedly due in part to contamination by a species of Penicillium which made its appearance during the last week of growth. The average hydrogen-ion concentration was at the conclusion of the experiment P[H] 7.35.

In order to determine whether or not the enzyme invertase is present in the culture solution, 500 cc. portions of the solutions were incubated for 14 days at a temperature of 32° C. As an antiseptic agent, 2 percent of

TABLE 2. *Corn. Duration, Dec. 31, 1918 to Feb. 10, 1919: 42 days.*

Culture Solution	Culture Number	Water Transpired (Cubic Centimeters)	Green Weight (Grams)	Dry Weight			Total Sugar at End of Experiment, Calculated as Sucrose (Grams)	Sugar Used, Calculated as Sucrose (Grams)	Reducing Sugar Present at End of Experiment (Grams)
				Roots (Grams)	Tops (Grams)	Total (Grams)			
Pfeffer's + sucrose...	6	300	21.5	0.220	0.970	1.19	3.769	0.984	0.583[1]
" + " ...	7	326	27.0	0.410	1.420	1.83	4.314	0.439	0.434
" + " ...	8	450	29.0	0.540	1.620	2.160	3.657	1.096	0.976[2]
" + " ...	9	340	29.0	0.290	1.30	1.590	4.217	0.535	0.3125
" + " ...	10	270	23.0	0.270	1.250	1.520	4.595	0.158	0.328
" + " ...		Control solution no plant					4.753		trace
Pfeffer's............	11	300	13.2	0.120	0.610	0.730			No reducing sugar
"	12	310	16.5	0.230	1.050	1.280			
"	13	190	11.5	0.220	0.650	0.870			
"	14	168	10.5	0.130	0.520	0.650			
"	15	300	20.0	0.190	1.100	1.290			

[1] Kept in laboratory in diffused light for 10 days before analysis.
[2] Contaminated.

toluene was used. At the end of the 14 days, analyses were again made for reducing sugars. No increase was shown in any case after incubation except in number 3. In this case the amount of reducing sugar had nearly doubled, due undoubtedly to the enzyme invertase derived from the Penicillium contamination.

In the Canada field pea cultures only one of the sucrose cultures remained uncontaminated. The duration of growth in this case was 50 days, the green weight 14.95 grams, and the amount of reducing sugar present 0.448 gram. The total sugar present calculated as sucrose was 3.711 grams, and the amount of sugar as sucrose used was 1.042 grams. The non-sucrose cultures did not show any reducing sugars in the culture medium. As in the experiment with corn, 500 cc. of the culture solution was incubated for 14 days at a temperature of 32° C. No increase in reducing sugar was found at the end of that period.

Experiment 3. A white dent variety of corn was used and the plants were grown as before in the greenhouse. The duration of the experiment was from June 27 to July 29, a period of 32 days. The concentration of sucrose was ½ percent. In this experiment the hydrogen-ion concentration of the culture solution was again accurately determined by the indicator method, using anhydrous KH_2PO_4 and Na_2HPO_4 according to Soerensen and using neutral red as the indicator. In addition to determining the hydrogen-ion concentration, several cultures were provided, to each of which was added 1 cc. of a ½ percent solution of neutral red, the purpose being to follow the reaction during plant growth. This was possible for only about ten days, for the plant by the tenth day had absorbed all the indicator. From the outset the solution became increasingly alkaline, so that it was only at the outset that an acid reaction prevailed and then the hydrogen-ion concentration was only $10^{-6.7}$ normal.

The results of experiment 3 are similar to those of the preceding experiments. There is the usual increase in reducing sugars; the reaction of the solution at the outset was very slightly acid (P[H] 6.7), and at the conclusion slightly alkaline (P[H] 7.25 to 7.3)

Incubation experiments were made as in the previous experiments.

TABLE 3. *Corn. Duration of experiment, 32 days.*

Culture Number	Sugar as Sucrose at End (Grams)	Sucrose Used by Plant (Grams)	Reducing Sugar at End (Grams)	Gain in Reducing Sugar (Grams)	P[H] at Conclusion of Experiment
16.........	4.891	0.940	0.476	0.376	7.3
17.........	4.550	1.181	0.400	0.300	7.30
18.........	4.912	0.819	0.375	0.275	7.25
19.........	4.912	0.819	0.571	0.471	7.25
Control, no plant......	5.731		0.100		6.70

The duration of the incubation experiment was 14 days. Toluene 2 percent was added as the antiseptic agent, and the temperature of incubation was 35° C. No increase in reducing sugar over that found in the culture solution was noted after the period of incubation.

Experiment 4. This experiment was like the preceding. Canada field pea was used and the results appear in table 4.

TABLE 4. *Canada field pea. Duration, Sept. 4 to Sept. 22: 18 days.*

Culture Solution	Culture Number	Dry Weight			Reducing Sugar Present	Gain in Reducing Sugars
		Roots (Grams)	Tops (Grams)	Total (Grams)		
Pfeffer's solution + sucrose.......	1	0.221	0.254	0.475	839	0.333
" " + "	2	0.186	0.322	0.508	877	0.371
" " + "	3	0.223	0.277	0.500	820	0.314
" " + "	4	0.200	0.260	0.460	876	0.370
" " + " .... no plant					506	
Pfeffer's solution.................	1	0.113	0.216	0.339	No reducing sugars in these solutions	
" "	2	0.127	0.309	0.436		
" "	3	0.083	0.169	0.252		
" "	4	0.099	0.169	0.268		

Experiment 5. Culture in distilled water. In order to determine whether or not the character o the nutrient solution had any special significance with respect to the increase in reducing sugars, an experiment was made using in place of Pfeffer's solution merely distilled water to which was added 1 percent sucrose. Canada field pea was used and the duration of the experiment was 18 days. The conditions and methods of the experiment were similar to those of the preceding experiments.

Only one culture remained uncontaminated. The green weight of the plant was 1.35 grams, the reducing sugar in the culture solution (1000 cc.) at the conclusion of the experiment was 293 milligrams, while in the control there was only 138 milligrams; there was an increase therefore of 155 milligrams in the culture solution.

An incubation experiment was also made. 500 cc. samples were taken from the culture solution and from the control solution, and toluene was added. The solutions were incubated at 35° C. and then again analyzed for reducing sugars. The increase after nine days was but 5 milligrams.

Nutrient solution minus iron salts. Rice and Osugi (8) in working on the inverting power of various soils have presented evidence that inversion of sucrose may be affected by various colloids and suggest that the inversion may be due to adsorbed acids. In the Pfeffer's solution after sterilization there is precipitated ferric hydrate, and this precipitate is increased after a few days' growth of the plant. In order to determine whether or not the ferric hydrate might be responsible for the increase in reducing sugar, an experiment was performed in which iron was omitted from the culture solution. The methods were the same as for the previous experiments. Sucrose was supplied at a concentration of 0.5 percent. Corn was again used and the plants were grown for 18 days in the greenhouse. The dry weights of tops and roots were 0.725 grams and 0.200 grams respectively. Analyses showed 500 milligrams of reducing sugars, while the control solution had only 305 milligrams. The increase was therefore 95 milligrams.

Discussion

What is the cause of the increase in reducing sugar in the culture medium? Is the enzyme invertase excreted? The evidence is contrary to this idea. In no case was there obtained any increase in reducing sugar after incubation. It is possible, of course, that the enzyme invertase is excreted from the root in such small amounts that the reaction effected is very slight. It might be suggested, furthermore, that the culture solution is unfavorable to the invertase and that the latter is soon destroyed. It was noted, however, that whenever the culture solution became contaminated with a yeast or a fungus, there was a marked increase in reducing sugars, and that this increase continued after incubation. The incubation experiment for culture number 8 of experiment 2 yielded data in support of this statement. In accordance with the view of Rice and Osugi (8) it might be expected that the mucilaginous matter of the root and surrounding the root-cap cells as well as the cell walls might adsorb basic ions, the process resulting in a preponderance of hydrogen ions which might cause inversion of sucrose. But since the culture solution becomes increasingly alkaline in reaction with the advent of time, and since this alkalinity is due to the absorption of anions by the roots, it is reasonable to conclude that the zone about the roots is constantly of greater alkalinity than the "outer" regions of the culture solutions. In other words, the gradient of concentration of hydroxyl ions falls with increasing distance from the roots.

There is still another alternative. The cells of the root-cap are sloughed off, and it might be suggested that the root cells in dying yield reducing sugar to the culture solution. But, as stated in another paper (Knudson, 5),

the writer has found that the root-cap cells that accumulate at the bottom of the culture flasks are not dead but apparently remain alive for a very considerable period. Examination of the sloughed off root-cap cells at the conclusion of the experiments revealed that they were alive and in good condition. Furthermore, the total weight of such cells would not be over 20 milligrams.

It seems to the writer that there is only one explanation to account for the accumulation of reducing sugars, and that is excretion of reducing sugars by the roots.

In accordance with this view, the procedure might be as follows: Sucrose is absorbed by the roots and inverted in the root cells by the enzyme invertase. Some of the sugar is used in growth, but there is a superabundance of reducing sugars and they accumulate in the root cells. At the outset there are practically no reducing sugars in the sucrose solutions. The concentration gradient between the reducing sugars in the cells and those outside is steep, and consequently some of the reducing sugars diffuse outward. With the progress of time the difference in concentration of reducing sugars becomes less, but probably it is considerable at all times, since at the conclusion of the experiment the concentration of the sucrose in the culture is much greater than that of the reducing sugars; and since there is a constant inward diffusion of sucrose, there results a constant production of reducing sugars in the plant cells.

In support of the view that the reducing sugars are excreted, the following experiment may be cited. Three corn plants which had grown for 30 days in Pfeffer's solution, each plant having a fresh weight of approximately 18 grams, were removed from the culture vessels and the roots washed in tap water. At 5 p.m. the plants were transferred to culture vessels so that their roots alone were bathed in a four percent solution of sucrose (Merck's highest purity). Three culture vessels were used and 400 cc. of the solution. The roots were kept in this solution for 16 hours. The plants were then removed and rinsed seven times in tap water, and then the plants were transferred to culture vessels this time containing distilled water. The plant roots remained in distilled water 7 hours. The total volume of distilled water was then reduced by evaporation to 100 cc. This was analyzed for reducing sugar and the determinations gave 14.5 milligrams of reducing sugar.

In another experiment plants were used which had been growing in a nutrient solution plus sucrose, and treated in the same way as in the preceding experiment. There were leached from the roots of four plants 75 milligrams of reducing sugar and 150 milligrams of sucrose.

The secretion of sugars by the roots of plants may seem at the outset to be a rather startling idea, yet theoretically there is no reason why this should not occur. Wächter (9) reported considerable excretion of sugars by slices of beets and onions when immersed in distilled water or in salt

solutions, and recently much evidence has been presented showing the leaching of electrolytes from the roots of plants (see Merrill (6) for a review o literature).

Summary and Conclusions

1. Evidence is presented to show that Canada field pea (*Pisum arvense* L.) and corn (*Zea mays* L.) grown in the presence of sucrose cause an increase in reducing sugars in the culture solution.

2. The reaction of the culture solution is such as to be without influence on the sucrose.

3. Incubation experiments yielded negative results with respect to the presence of invertase.

4. The idea is held that the increase in reducing sugars is due to excretion of these from the roots.

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